Molecular marker diversity of SCN-resistant sources in soybean

Yiwu Chen, Dechun Wang, Prakash Arelli, Mohsen Ebrahimi, and Randall L. Nelson

Abstract: Soybean cyst nematode (SCN) (*Heterodera glycines* Ichinohe; HG) is one of the most destructive pests of soybean (*Glycine max* (L.) Merr.) in the United States. Over 100 SCN-resistant accessions within the USDA Soybean Germplasm Collection have been identified, but little is known about the genetic diversity of this SCN-resistant germplasm. The objective of this research was to evaluate the genetic variation and determine the genetic relationships among SCN-resistant accessions. One hundred twenty-two genotypes were evaluated by 85 simple sequence repeat (SSR) markers from 20 linkage groups. Non-hierarchical (VARCLUS) and hierarchical (Ward's) clustering were combined with multidimensional scaling (MDS) to determine relationships among tested lines. The 85 SSR markers produced 566 allelic fragments with a mean polymorphic information content (PIC) value of 0.35. The 122 lines were grouped into 7 clusters by 2 different clustering methods and the MDS results consistently corresponded to the assigned clusters. Assigned clusters were dominated by genotypes that possess one or more unique SCN resistance genes and were associated with geographical origins. The results of analysis of molecular variance (AMOVA) showed that the variation differences among clusters and individual lines were significant, but the differences among individuals within clusters were not significant.

Key words: soybean cyst nematode resistance, genetic diversity, SSR markers, cluster analysis, soybean.

Résumé : Le nématode à kystes du soya (SCN ; *Heterodera glycines* Ichinohe; HG) représente un des ravageurs les plus importants chez le soya (*Glycine max* (L.) Merr.) aux États-Unis. Plus de 100 accessions résistantes ont été identifiées au sein de la collection du USDA Soybean Germplasm Collection, mais peu de choses sont connues au sujet de la diversité parmi ce germoplasme résistant au SCN. L'objectif de cette recherche était d'évaluer la variation génétique et de déterminer les relations génétiques entre les accessions résistantes au SCN. Cent vingt-deux génotypes ont été évalués à l'aide de 85 microsatellites (SSR) couvrant les 20 groupes de liaison. Des analyses de groupement non-hiérarchiques (VARCLUS) et hiérarchiques (de Ward) ont été combinées avec l'échelonnement multidimensionnel (MDS) afin de déterminer les relations entre les lignées testées. Les 85 microstellites ont produit 566 allèles et un indice PIC moyen de 0,35. Les 122 lignées ont été assemblées en sept groupes au moyen de deux méthodes de groupement et les résultats MDS concordaient bien à ces groupements. Les groupes étaient dominés par des génotypes possédant un ou plusieurs gènes de résistance au SCN et ils étaient corrélés à l'origine géographique. Les résultats AMOVA ont montré que les différences de variation entre groupes et lignées individuelles étaient significatives, mais les différences entre individus au sein des groupes n'étaient pas significatives.

Mots clés : résistance au nématode à kystes du soya, diversité génétique, microsatellites, analyse de groupement, soya.

[Traduit par la Rédaction]

Introduction

Soybean cyst nematodes (SCN), *Heterodera glycines* Ichinohe (HG), is the most important pest of soybean (*Glycine max* (L.) Merr.) in the United States. Using host plant resistance is the most effective way of controlling the damage caused by SCN in soybean. Over 100 accessions in the USDA Soybean Germplasm Collection have been identified as resistant to SCN (Anand and Gallo 1984; Anand et al. 1985; Anand et al. 1988; Arelli et al. 2000; Epps and Hartwig 1972; Rao Arelli et al. 1997; Ross and Brim 1957; Young 1990; Young 1995). However, only a few resistance sources have been used extensively in the development of SCN-resistant cultivars. Genetic allelism and quantitative trait locus (QTL) mapping studies have demonstrated that SCN-resistant accessions used in cultivar development have major resistance genes in common (Concibido et al. 1996, 1997; Arelli et al. 1992; Webb et al. 1995). As a result, the genetic diversity of SCN resistance in commercial cultivars is even more limited than would be indicated by the few resistant sources used. Increasing available genetic diversity for SCN resistance is critical for the long-term stability of host plant resistance as a control strategy for SCN. Prior knowledge of genetic

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relationships among SCN-resistant sources will facilitate the development of new cultivars with novel resistance genes and enhance the genetic base of SCN resistance in these cultivars. Molecular markers were useful tools to estimate genetic relationships among SCN-resistant accessions. Estimation of genetic diversity of soybean lines resistant to SCN has been conducted in several studies. After surveying the SCN-resistant lines with RFLP markers, Zhang et al. (1999) found that there were 2 major resistance resources among 56 genotypes tested in the study; one from Korea and the other from China. Diers et al. (1997) used RFLP markers to characterize 38 plant introductions (PI) previously reported as being resistant to one or more SCN races. Based on cluster and principal component analysis, the grouping of the PIs was associated with resistance responses to the races of *H. glycines* and one major group of PIs was distinct from all previously used resistant sources. The authors believed that those PIs could provide soybean breeders with a novel resistant source for the development of improved cultivars.

The objectives of this research were to quantify the genetic variation among 122 PI lines and cultivars using SSR markers, to examine the genetic relationships among tested genotypes, and to investigate the relationships between geographical origin and SCN resistance.

Materials and methods

One hundred twenty-two lines including PIs and cultivars were selected from the USDA Soybean Germplasm Collection with resistance to various combinations of races 1, 2, 3, 5, or 14 (corresponding to HG types 2, 1.2, 0, 2, and 1.3, respectively; Niblack et al. 2002). One wild soybean *(Glycine soja)* line (PI 468916) and 3 susceptible cultivars ('BSR101', 'Dunfield', and 'Hutcheson') were used for comparison in this study (Table 1). These accessions generally originated from northeast and central China, Korea, Japan, and far eastern Russia and all lines are in US maturity groups 0 to VII (Table 1). Seeds of the tested lines were from the USDA Soybean Germplasm Collection. The SCN resistance ratings of the tested lines were compiled from published data (Table 1). All lines were grown in a greenhouse at Michigan State University in May 2005 for DNA extraction.

Genomic DNA of the tested lines was isolated from the first trifoliate leaves of 10 greenhouse-grown seedlings of each accession using the CTAB (hexadecyltrimethylammonium bromide) method of Kisha et al. (1997). The DNA concentration of all samples was adjusted to a concentration of 25 ng/μL for polymerase chain reaction (PCR). The PCR was performed according to Cornelious et al. (2005). Amplified products were separated using 6% *w/v* non-denaturing polyacrylamide gel with a vertical sequencing system described by Wang et al. (2003).

Eighty-five SSR markers were selected from the consensus soybean linkage map (Song et al. 2004) to evaluate genetic relationships among SCN-resistant PI lines. These SSR markers cover all of the 20 linkage groups on the consensus map and some of the SSR markers are associated with QTLs underlining SCN resistances and other traits in soybean (Table 2). The sequences of the primers were obtained from Dr. Perry Cregan, USDA–ARS at Beltsville, Md., and were synthesized at the Genomics Technology Support Facility of Michigan State University.

SSR alleles were classified by molecular mass. Because SSR is a codominant marker, a genetic dissimilarity coefficient was calculated as 1 (total score/total loci), where "total loci" is the total number of SSR loci and "total score" is the sum of values over those loci (Diwan and Cregan 1997). For a pair of genotypes if both alleles were identical at a locus the score is 1.0, if only 1 allele was identical the score is 0.5; and if no alleles were identical the score is 0.

A hierarchical cluster analysis method was performed on the 122 × 122 genetic dissimilarity coefficient matrix using the "WARD" option of PROC CLUSTER of PC SAS (SAS Institute Inc. 1999). By setting the TRIM function to 1, 2% of the lines possessing low probability estimates were removed. The matrix of genetic dissimilarity coefficient was also subjected to multidimensional scaling (MDS) (Shepard 1974) by the MDS procedure in PC SAS (SAS Institute Inc. 1999). The "absolute" option was applied to maintain the scale of 0 and 1 for ease of interpretation and graphing. A nonhierarchical cluster analysis procedure was also employed to the original allelic data to divide the lines into nonoverlapping clusters by using the PROC VARCLUS option of SAS (SAS Institute Inc. 1999).

Gene diversity over loci, so called polymorphism information content (PIC) scores (Anderson et al. 1992), can be estimated by the following formula (Nei 1987):

$$\hat{H} = \left(\frac{n}{n-1}\right)\left(1 - \sum_{i=1}^{k} p_i^2\right)$$

Its sampling variance also can be estimated as follows:

$$V(\hat{H}) = \frac{2}{n(n-1)} \times \left\{2(n-2)\left[\sum_{i=1}^{k} p_i^3 - (\sum_{i=1}^{k} p_i^2)^2\right] + \sum_{i=1}^{k} p_i^2 - (\sum_{i=1}^{k} p_i^2)^2\right\}$$

where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the ith haplotype.

The analysis of molecular variance (AMOVA) procedure in ARLEQUIN version 3.0 (Excoffier et al. 2005) was used to estimate the components of variance, which is attributable to differences among clusters and among individuals within clusters, based on the genetic distance matrix, as specified in the analysis of molecular variance. Significance of variance components associated with the different possible levels of genetic structure in this study was tested by a nonparametric permutation procedure with 1023 permutations (Excoffier at al. 1992). The F statistic, F_{st} value, was used to estimate the correlation of genes of different individuals in the same population and to measure the genetic differences among subpopulations. If the subpopulations were identical in all allele frequencies, then F_{st} equaled 0; if the subpopulations were fixed for different alleles, then F_{st} equaled 1. Therefore, pairwise comparisons of values of F_{st} among populations were considered standardized interpopulation distances between populations.The pairwise F_{st} values, a value of F

Table 1. The 122 genotypes used in this study and their responses to SCN race 1, 2, 3, 5, and 14.

Code	Accessions	Origin	MG[a]	Seed color	Responses to different races[b]					Ward cluster	VARCLUS cluster
					Race 1	Race 2	Race 3	Race 5	Race 14		
S001	BSR 101[c]	USA	I	Yellow	S	S	S	S	S	I	7
S002	'Dunfield'[c]	USA	III	Yellow	S	S	S	S	S	I	7
S003	'Essex'[c]	USA	V	Yellow	S	S	S	S	S	I	7
S004	'Forrest'[c]	USA	V	Yellow	R	S	R	S	S	I	7
S005	'Hartwig'[c]	USA	V	Yellow	R	R	R	R	R	I	7
S006	'Hill'[d,j]	USA	V	Yellow	NA	NA	MS	S	NA	I	7
S007	'Hutcheson'[c]	USA	V	Yellow	S	S	S	S	S	II	7
S008	'Ilsoy'[d,e]	USA	III	Brown	MR	S	MR	MR	MR	II	2
S013	PI 548316[d,e]	Zhejiang, China	III	Black	MS	S	R	S	MR	II	2
S009	'Ina'[f]	USA	IV	Yellow	R	R	R	R	MR	II	7
S010	'Kenwood'[c]	USA	II	Yellow	S	S	S	S	S	II	7
S011	'Loda'[g]	USA	II	Yellow	MR	R	R	MR	MR	II	7
S015	PI 209332[c,d]	Japan	IV	Black	MS	S	R	R	R	II	2
S022	PI 399061[d]	South Korea	VI	Yellow	NA	NA	MR	R	NA	II	4
S029	PI 416762[d]	Japan	II	Black	MR	NA	R	R	R	II	4
S033	PI 424595[d]	South Korea	VI	Black	NA	NA	NA	R	NA	II	2
S034	PI 437090[d]	Russia	O	Yellow	NA	NA	NA	MS	MS	II	4
S042	PI 437908[d]	Northeast China	II	Yellow	NA	NA	MR	NA	NA	II	4
S036	PI 437488[d]	China	II	Black	NA	NA	NA	NA	MR	II	4
S016	PI 548317[d,e]	Hebei, China	III	Green	MR	S	R	MR	R	II	2
S017	PI 548389[c]	USA	0	Yellow	S	S	S	S	S	II	2
S020	PI 398680[d]	South Korea	IV	Green	NA	NA	MR	NA	NA	II	4
S021	PI 398682[d]	South Korea	IV	Black	NA	NA	MR	NA	NA	II	4
S028	PI 408192–2[d]	South Korea	V	Green	MS	NA	NA	MS	MS	II	4
S027	PI 407944[d]	South Korea	V	Yellow	NA	NA	MR	NA	NA	II	4
S032	PI 424137B[d]	South Korea	V	Yellow	NA	NA	NA	R	MR	II	4
S035	PI 437379[d]	Russia	I	Yellow	NA	NA	NA	MS	NA	II	4
S012	'Peking'[c]	China	IV	Black	R	S	R	R	MS	III	2
S019	PI 339868B[d]	South Korea	IV	Black	R	NA	R	R	NA	III	2
S023	PI 404166[d]	China	III	Black	R	R	R	R	NA	III	2
S014	PI 200495[d]	Japan	IV	Black	MR	NA	MR	R	MR	III	2
S026	PI 407729[d]	China	IV	Black	MR	NA	MR	R	R	III	2
S018	PI 303652[d]	China	V	Black	R	NA	R	R	MR	III	2
S025	PI 404198B[d]	China	IV	Black	R	NA	R	R	MR	III	2
S024	PI 404198A[d]	China	IV	Black	R	R	R	R	NA	III	2
S030	PI 417091[d]	Japan	II	Black	MR	NA	R	R	R	III	2
S037	PI 437654[c]	China	III	Black	R	R	R	R	R	III	2
S031	PI 417094[d]	China	III	Black	NA	NA	MR	MR	MR	III	2
S046	PI 438496B[d]	Unknown	III	Black	MR	NA	R	NA	NA	III	2
S038	PI 437679[d]	China	IV	Black	MR	MR	R	R	R	III	2
S039	PI 437690[d]	China	III	Black	R	R	R	R	MR	III	2
S040	PI 437725[d]	China	IV	Black	R	MR	R	R	NA	III	2
S047	PI 438497[d]	Unknown	III	Black	R	NA	NA	R	NA	III	2
S048	PI 438498[d]	Unknown	IV	Black	R	NA	R	R	NA	III	2
S041	PI 437770[e,j]	China	III	Black	MS	S	R	S	NA	III	2
S044	PI 438342[d]	Argentina	VI	Black	NA	MR	NA	R	MR	III	2
S045	PI 438489B[c]	Unknown	IV	Black	R	R	R	R	R	III	2
S043	PI 438183[d]	Northeast China	II	Brown	NA	NA	MR	NA	NA	III	2
S049	Pickett 71[c]	USA	VI	Yellow	R	S	R	S	S	IV	4
S062	PI 468916[j,k]	Liaoning, China	III	Black	MS	NA	R	NA	NA	IV	3
S107	PI 567583C[c]	Shandong, China	IV	Yellow	MS	S	S	S	R	IV	1
S108	PI 567583D[c]	Shandong, China	IV	Yellow	S	S	R	S	S	IV	1
S117	PI 89008[d]	China	II	Green	NA	NA	MR	MR	MR	IV	1
S118	PI 89014[d]	China	II	Yellow	MS	NA	MS	MS	MS	IV	1
S099	PI 567510A[c]	Hebei, China	III	Yellow	S	S	S	S	R	IV	1

Table 1. (*continued*).

Code	Accessions	Origin	MG[a]	Seed color	Responses to different races[b]					Ward cluster	VARCLUS cluster
					Race 1	Race 2	Race 3	Race 5	Race 14		
S100	PI 567512B[c]	Hebei, China	II	Yellow	S	S	S	S	R	IV	1
S101	PI 567516C[c]	Shandong, China	IV	Green	R	MR	R	S	S	IV	1
S104	PI 567568A[c]	Shandong, China	IV	Yellow	MS	S	R	S	R	IV	1
S102	PI 567535A[c]	Shandong, China	IV	Yellow	S	S	S	NA	R	IV	1
S103	PI 567562A[c]	Shandong, China	IV	Yellow	S	S	R	S	S	IV	1
S105	PI 567577[c]	Shandong, China	IV	Yellow	S	S	R	S	S	IV	1
S106	PI 567581[c]	Shandong, China	IV	Yellow	S	S	S	S	R	IV	1
S109	PI 567636[c]	Henan, China	IV	Yellow	S	S	R	MS	S	IV	1
S114	PI 84751[e,j]	South Korea	IV	Black	R	MS	R	S	NA	IV	1
S110	PI 567660B[c]	Henan, China	V	Yellow	S	MS	R	S	S	IV	1
S113	PI 79693[e,j]	Heilongjiang, China	III	Brown	MR	S	MR	MS	NA	IV	1
S116	PI 88788[c]	China	III	Black	S	S	R	S	R	IV	1
S119	PI 89772[d]	China	IV	Black	R	R	R	R	NA	IV	1
S120	PI 90763[c]	China	IV	Black	R	R	R	R	MS	IV	1
S121	PI 91138[d]	China	II	Yellow	MS	NA	MS	MS	MS	IV	1
S111	PI 548400[j]	Heilongjiang, China	IV	Yellow	S	S	MS	S	NA	IV	1
S115	PI 87631–1[d]	Japan	III	Green	NA	NA	R	MR	MR	IV	1
S112	PI 79609[d]	China	II	Black	NA	NA	MR	NA	NA	IV	1
S122	PI 92720[d]	China	III	Black	NA	NA	MR	NA	NA	IV	1
S050	'Sooty'[d]	China	IV	Black	NA	NA	MR	MR	MR	V	3
S051	PI 438503A[d]	Unknown	II	Black	NA	NA	R	MR	R	V	3
S052	PI 458519AH[i]	Jilin, China	II	Black	MS	MR	MR	MR	MR	V	3
S054	PI 461509[h,i]	Jilin, China	I	Brown	MS	MR	R	MR	MR	V	3
S055	PI 464915B[h,i]	Jilin, China	II	Black	MS	MR	MR	MR	MR	V	3
S053	PI 458520[h,i]	Jilin, China	II	Brown	MS	MS	R	R	MR	V	3
S057	PI 467312[c]	Jilin, China	II	Green	S	MS	R	R	R	V	3
S059	PI 467332[h,i]	Jilin, China	II	Green	MS	MS	R	MR	R	V	3
S056	PI 467310[h,i]	Jilin, China	II	Yellow	S	MS	MR	MR	MR	V	3
S058	PI 467327[h,i]	Jilin, China	II	Brown	MS	R	MS	R	MS	V	3
S064	PI 490769[h,i]	Hebei, China	III	Black	MS	MR	MR	MR	MR	V	3
S060	PI 468903[h,i]	Jilin, China	II	Black	MR	R	R	R	S	V	3
S061	PI 468915[h,i]	Liaoning, China	II	Black	R	MR	R	R	S	V	3
S072	PI 532434[h,i]	Jilin, China	II	Black	MS	MR	MR	MS	MR	V	3
S073	PI 532444A[h,i]	Jilin, China	I	Brown	S	MR	MR	MR	MR	V	3
S074	PI 532444B[h,i]	Jilin, China	II	Brown	MS	MR	MS	MS	MR	V	3
S063	PI 475810[h,i]	Xinjiang, China	II	Yellow	MS	MS	S	MR	MS	VI	3
S079	PI 567303A[c]	Gansu, China	IV	Black	S	NA	S	S	R	VI	6
S083	PI 567336A[c]	Gansu, China	IV	Black	S	MR	R	R	S	VI	6
S084	PI 567336B[c]	Gansu, China	IV	Black	MS	MR	R	R	S	VI	6
S085	PI 567342[c]	Gansu, China	V	Green	MR	R	R	R	S	VI	6
S065	PI 494182[h,i]	Japan	0	Yellow	R	MR	R	R	MS	VI	6
S070	PI 507476[h,i]	Japan	VI	Yellow	MR	MS	R	R	MS	VI	6
S066	PI 495017C[h,i]	Beijing, China	IV	Green	MS	MR	R	MS	MR	VI	6
S069	PI 507471[h,i]	Japan	III	Yellow	MR	R	MS	R	MR	VI	6
S075	PI 5459[d]	China	III	Yellow	S	S	NA	MR	NA	VI	6
S076	PI 561395	Japan	IV	Yellow	NA	NA	NA	NA	NA	VI	6
S081	PI 567325B[e,j]	Gansu, China	V	Yellow	S	S	R	NA	NA	VI	6
S082	PI 567328[c]	Gansu, China	V	Yellow	S	S	S	S	R	VI	6
S077	PI 567285[c]	Gansu, China	III	Yellow	S	S	R	S	S	VI	6
S078	PI 567286[c]	Gansu, China	III	Yellow	S	NA	R	S	R	VI	6
S067	PI 507422[h,i]	Japan	VI	Yellow	R	MS	R	MS	S	VI	6
S068	PI 507443[h,i]	Japan	IV	Yellow	MR	S	R	MR	MS	VI	6
S071	PI 509100[h,i]	South Korea	VII	Green	R	MS	R	MR	S	VI	6
S080	PI 567325[c]	Gansu, China	II	Yellow	S	S	R	S	S	VII	5
S086	PI 567363B[c]	Ningxia, China	III	Yellow	S	S	R	S	S	VII	5

Table 1. (*concluded*).

Code	Accessions	Origin	MG[a]	Seed color	Responses to different races[b]					Ward cluster	VARCLUS cluster
					Race 1	Race 2	Race 3	Race 5	Race 14		
S087	PI 567364[c]	Ningxia, China	III	Yellow	S	S	R	MS	R	VII	5
S088	PI 567365[c]	Ningxia, China	III	Green	S	S	R	MS	S	VII	5
S090	PI 567373B[c]	Ningxia, China	V	Yellow	MS	MS	S	MS	R	VII	5
S089	PI 567373A[c]	Ningxia, China	IV	Yellow	S	S	S	MR	R	VII	5
S091	PI 567400[c]	Shannxi, China	V	Yellow	S	S	S	S	R	VII	5
S092	PI 567415A[c]	Shannxi, China	IV	Yellow	MR	MS	S	MR	R	VII	5
S093	PI 567418A[c]	Shannxi, China	II	Yellow	S	S	S	S	R	VII	5
S094	PI 567421[c]	Shannxi, China	IV	Yellow	S	S	S	S	R	VII	5
S096	PI 567491A[c]	Hebei, China	III	Black	R	MR	R	R	S	VII	5
S095	PI 567445B[c]	Shanxi, China	IV	Yellow	MR	MS	S	NA	R	VII	5
S097	PI 567492[c]	Hebei, China	IV	Yellow	S	S	S	S	R	VII	5
S098	PI 567507B[c]	Hebei, China	II	Yellow	S	S	S	S	R	VII	5

[a]MG, maturity group.
[b]S, susceptible; MS, moderately susceptible; MR, moderately resistant; R, resistant; NA, not available.
[c]Xie et al. 1998.
[d]Zhang et al. 1999.
[e]Arelli et al. 1997
[f]Nickell et al. 1999.
[g]Nickell et al. 2001.
[h]Arelli et al. 2000.
[i]Young 1995.
[j]USDA online Germplasm Resources Information Network, http://www.ars-grin.gov/index.html.
[k]Wang et al. 2001.

statistic analogues computed from AMOVA, were used for pairwise comparisons among clusters (Excoffier et al. 2005). Since SSR markers are codominant markers, the variance of allele frequencies was actually partitioned but not the genotypic variance as for dominant markers.

Results

SSR marker diversity

Eighty-five pairs of SSR primers generated 566 allelic fragments among 122 tested genotypes ranging from 100 to 600 bp. The number of allelic fragments produced by an SSR primer pair ranged from 2 to 13 alleles with an average of 6.7 (Table 2). Sat_123, Satt368, Satt579, and Satt665 had the least alleles (2) and Satt172 had the most alleles (13). Genetic diversity for a specific locus can be evaluated by polymorphic information content (PIC) scores. The higher the PIC score, the higher the probability that polymorphism will exist between 2 PIs at that locus. The range of PIC scores for polymorphic alleles was 0.02 (Satt300) to 0.50 (Satt130, Satt242, Satt343, and Satt385) with a mean of 0.35.

Cluster analysis

The CCC, PSF, and PST2 statistics from the output of PROC CLUSTER were examined to define the number of clusters generated on the basis of the SSR data. All 3 statistics indicated that there were 7 clusters among the 122 lines. Therefore, all 122 lines were assigned to 7 clusters by the Ward's minimum variance cluster analysis of the PROC CLUSTER procedure (Table 1). The nonhierarchical cluster analysis procedure, PROC VARCLUS, generated as many as 24 clusters, in which it explained 61% of the total variation. However, when 7 clusters were formed, it accounted for 42% of the total variation. The Ward 7-cluster scheme was adopted to compare with the clusters derived from VARCLUS. Cluster I contained 6 developed varieties in which 3 of them (BSR 101, Dunfield, and Essex) were susceptible to SCN. Both clustering procedures agreed on this cluster. In cluster II, there were 21 lines including 5 cultivars, 'Hutcheson', 'Ilsoy', 'Ina', 'Kenwood', and 'Loda'. These 5 cultivars were clustered as a subgroup. PI 209332 was clustered into cluster II in this study and was the only line genetically unrelated to most sources for SCN resistance (Figs. 1 and 2). PI 209332 was resistant to SCN races 3, 5, and 14. There were 21 lines in cluster III including 3 well-known resistant sources, 'Peking', PI 437654, and PI 438489B. Both PI 437654 and PI 438489B were resistant to race 1, 2, 3, 5, and 14 and are in maturity groups III and IV, respectively. These accessions were obtained from Russia with records indicating that the former was from China and that the latter was the American 'Chiquita'. 'Chiquita' was the name given to a soybean introduction that came from Wuhan, Hubei, in 1910 (Morse and Cartter 1939) but was lost before the USDA soybean collection was established in 1949. 'Chiquita' was described as having a yellow seed coat and PI 438489B has a black seed coat. If 'Chiquita' was sent to Russia, what was returned was not 'Chiquita' and the origin of PI 438489B is therefore unknown. Two clustering methods consistently assigned these 21 lines to the same group. Cluster IV contained 26 lines including 4 important SCN-resistant sources, PI 468916, PI 88788, PI 89772, and PI 90763. PI 88788, PI 89772, and PI 90763 were clustered in the primary subgroup, indicating that they had a very similar genetic background (Fig. 1). Both cluster procedures highly agreed on this cluster except PI 468916 and 'Pickett', which were

Table 2. The 85 SSR markers used in this study, their map positions and linked putative SCN QTLs, and the number of allelic fragments generated in the 122 lines.

SSR	LG[a]	Position[b]	Linked putative SCN QTL locations (cM)[b]	No. of allelic fragments	References
Satt276	A1	17.2		5	
Satt300	A1	30.9		7	
Satt385	A1	64.7		10	
Satt236	A1	93.2		5	
Satt177	A2	36.8		7	
Satt424	A2	60.6	48.3–60.6	7	Theor. Appl. Genet. 111: 965–971
Satt329	A2	110.9		8	
Satt409	A2	145.6		9	
Satt197	B1	46.4		7	
Satt415	B1	82.9		8	
Satt583	B1	84.2	65.0–84.2	6	Theor. Appl. Genet. 102: 921–928
Satt665	B1	96.4		2	
Sat_123	B1	100.9	84.0–101	2	Theor. Appl. Genet. 102: 921–928
Satt453	B1	124.0	102.6–124	6	Theor. Appl. Genet. 113: 1167–1173
Satt577	B2	6.0		4	
Satt168	B2	55.2	55.2–63	11	Theor. Appl. Genet. 102: 921–928
Satt534	B2	87.6		6	
Satt565	C1	0.0		6	
Satt396	C1	24.1	21.0–24.1	5	Theor. Appl. Genet. 102: 921–928
Satt194	C1	26.4		7	
Satt294	C1	78.6		5	
Satt180	C1	127.8		7	
Satt281	C2	40.3		7	
Satt307	C2	121.3		7	
Satt202	C2	126.2	126.2–145.5	6	Theor. Appl. Genet. 102: 921–928
Satt371	C2	145.5	126.2–145.5	6	Theor. Appl. Genet. 102: 921–928
Satt357	C2	151.9		6	
Satt179	D1a	64.7		7	
Satt368	D1a	43.8	43.8–48.1	5	Crop Sci. 41: 1589–1595
Satt342	D1a	48.1	43.8–48.1	2	Crop Sci. 41: 1589–1595
Satt184	D1a	103.4		10	
Satt157	D1b	37.1		11	
Satt266	D1b	59.6		8	
Satt141	D1b	72.9		10	
Satt579	D1b	75.9		2	
Satt172	D1b	100.9		13	
Satt271	D1b	137.1		4	
Satt372	D2	39.3	15.0–39.3	9	Crop Sci. 41: 1589–1595
Satt002	D2	47.7		4	
Satt226	D2	85.1		6	
Satt082	D2	87.2	86.2–88.2	4	Theor. Appl. Genet. 102: 91–96
Satt186	D2	105.5		8	
Satt411	E	12.9		6	
Satt268	E	44.3		8	
Satt452	E	45.1	37.3–45.1	7	Theor. Appl. Genet. 102: 921–928
Satt231	E	70.2	51.0–70.2	8	Crop Sci. 41: 1589–1595
Satt114	F	63.7		8	
Satt510	F	71.4		5	
Satt554	F	111.9		5	
Satt038	G	1.8	0.8–2.8	4	Crop Sci. 39: 982–987
Satt130	G	23.1	23.1–66.6	5	Theor. Appl. Genet. 102: 921–928
Satt324	G	33.3		7	
Satt199	G	62.2		3	
Satt012	G	66.6	62.2–66.6	6	Theor. Appl. Genet. 102: 921–928
Satt288	G	76.8		6	

Table 2. *(concluded)*.

SSR	LG[a]	Position[b]	Linked putative SCN QTL locations (cM)[b]	No. of allelic fragments	References
Satt472	G	94.8		7	
Satt191	G	96.6		7	
Satt353	H	8.5		7	
Satt192	H	44.0		6	
Satt253	H	67.2		7	
Satt434	H	105.7		7	
Satt419	I	21.9		11	
Satt354	I	46.2		7	
Satt292	I	82.8		7	
Satt440	I	112.7		6	
Satt249	J	12.3		6	
Satt414	J	37.8		8	
Satt431	J	78.8		7	
Satt242	K	14.4		7	
Satt441	K	46.2		8	
Satt196	K	104.8		8	
Satt588	K	117.0		2	
Satt143	L	30.2		4	
Satt156	L	56.1		9	
Satt373	L	107.2		8	
Satt590	M	7.8		7	
Satt175	M	67.0		8	
Satt308	M	130.8		9	
Satt009	N	28.5		3	
Satt022	N	102.1		4	
Satt358	O	5.4		7	
Satt259	O	39.8		6	
Satt173	O	58.4		12	
Satt592	O	100.4		5	
Satt243	O	119.5		7	

[a]Linkage group as defined in the soybean consensus map (Song et al. 2004).
[b]Position on the soybean consensus map (Song et al. 2004).

included into VARCLUS clusters 3 and 4, respectively. Clusters V, VI, and VII each contained 16, 18, and 14 lines, respectively. Clusters V, VI, and VII consistently agreed with VARCLUS clusters 3, 6, and 5, respectively. Most of the lines within these 3 clusters were recently introduced from China with a few from Japan (Table 1). PI 494182 in cluster VI, a recently identified new SCN-resistant source, is genetically very distinct from other resistant sources in the study.

Multidimensional scaling A plot of the first 2 dimensions of the multidimensional scaling was produced to illustrate genetic variation among the tested lines and to compare with the results of hierarchical and nonhierarchical cluster methods (Fig. 2). The first and second dimensions accounted for 60.5% and 19.0% of total variation, respectively, when 10 dimensions were applied in the solution to have less than 2% stress, a measure comparing original dissimilarity to the amount of mismatched distance generated from MDS. All 122 lines were evenly spread on the MDS plot. In general, clusters I and IV were located in the upper left quadrants; clusters II and III were positioned in the upper right quadrants; cluster V remained in the lower right quadrant; and clusters VI and VII were placed in the lower right quadrants. The commonly used SCN-resistant sources, PI 2093332 (S015), 'Peking' (S012), PI 437654 (S037), PI 438489 B (S045), PI 88788 (S116), PI 89772 (S119), and PI 90763 (S120) were placed in the upper plot, whereas PI 468916 (S062) was located in the lower quadrant of the MDS plot. The positioning of clusters in the 2-dimensional MDS plot was consistent with the assignment by Ward's minimum variance method and VARCLUS.

AMOVA to partition genetic variance among the clusters

The results of AMOVA showed that the differences among clusters and among individual lines of different clusters were significant, but the differences among individuals within clusters were not significant (Table 3). The largest variation was found among individuals of different clusters, accounting for 91.7% of the total variance. Only 0.13% of total variance was contributed by individuals within clusters, indicating that lines clustered in the same group were genetically very similar. Although only 8.14% of total variance was observed among clusters, it was significant.

The cluster pairwise distances (F_{st} values) ranged from 0.05 between cluster VI and VII to 0.17 between cluster I and V (Table 4). All the pairwise comparisons between clusters

Fig. 1. Dendrogram of the 122 lines with SCN resistance based on the genetic dissimilarity coefficient of 566 SSR allelic fragments with Ward's minimum variance method.

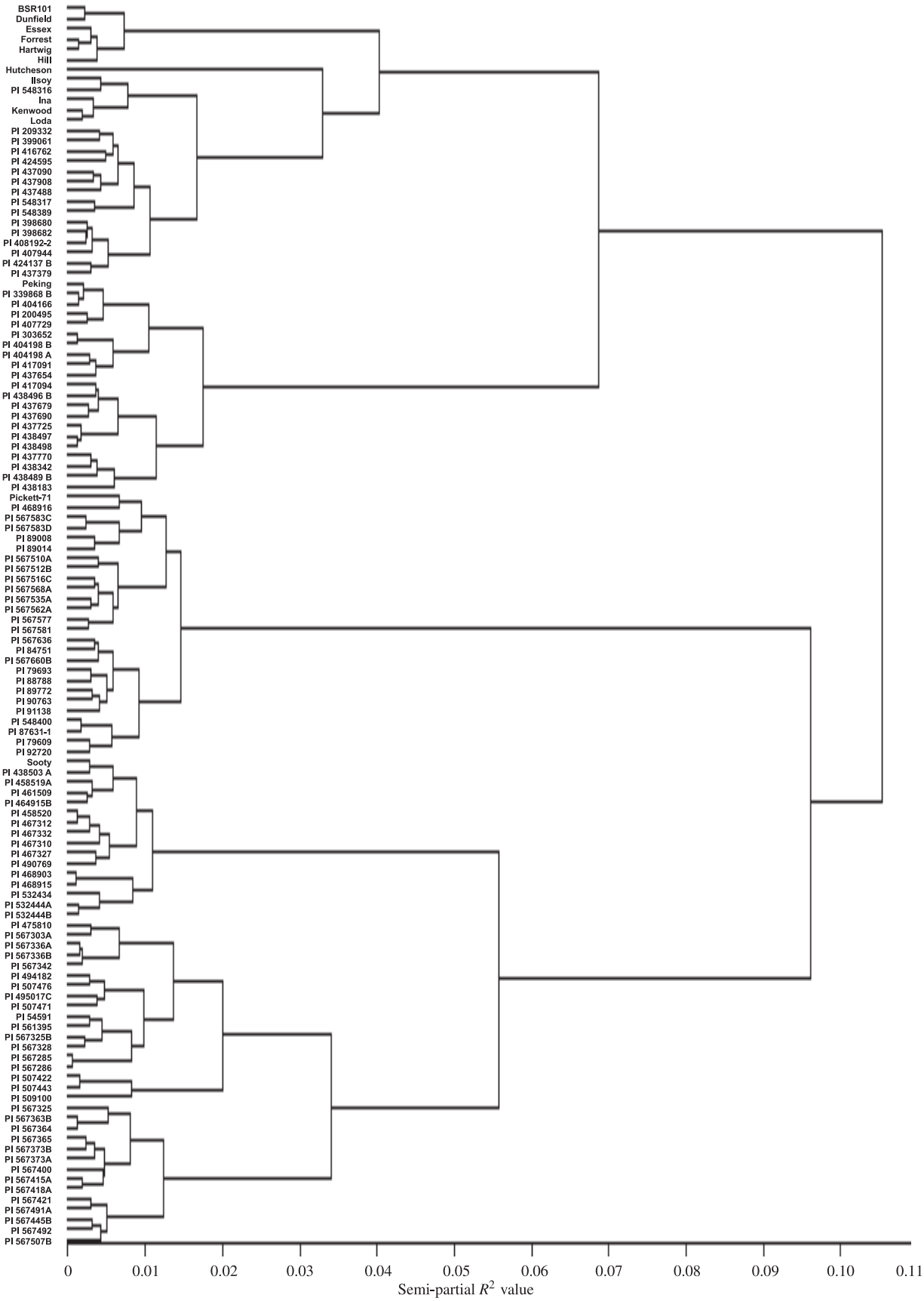
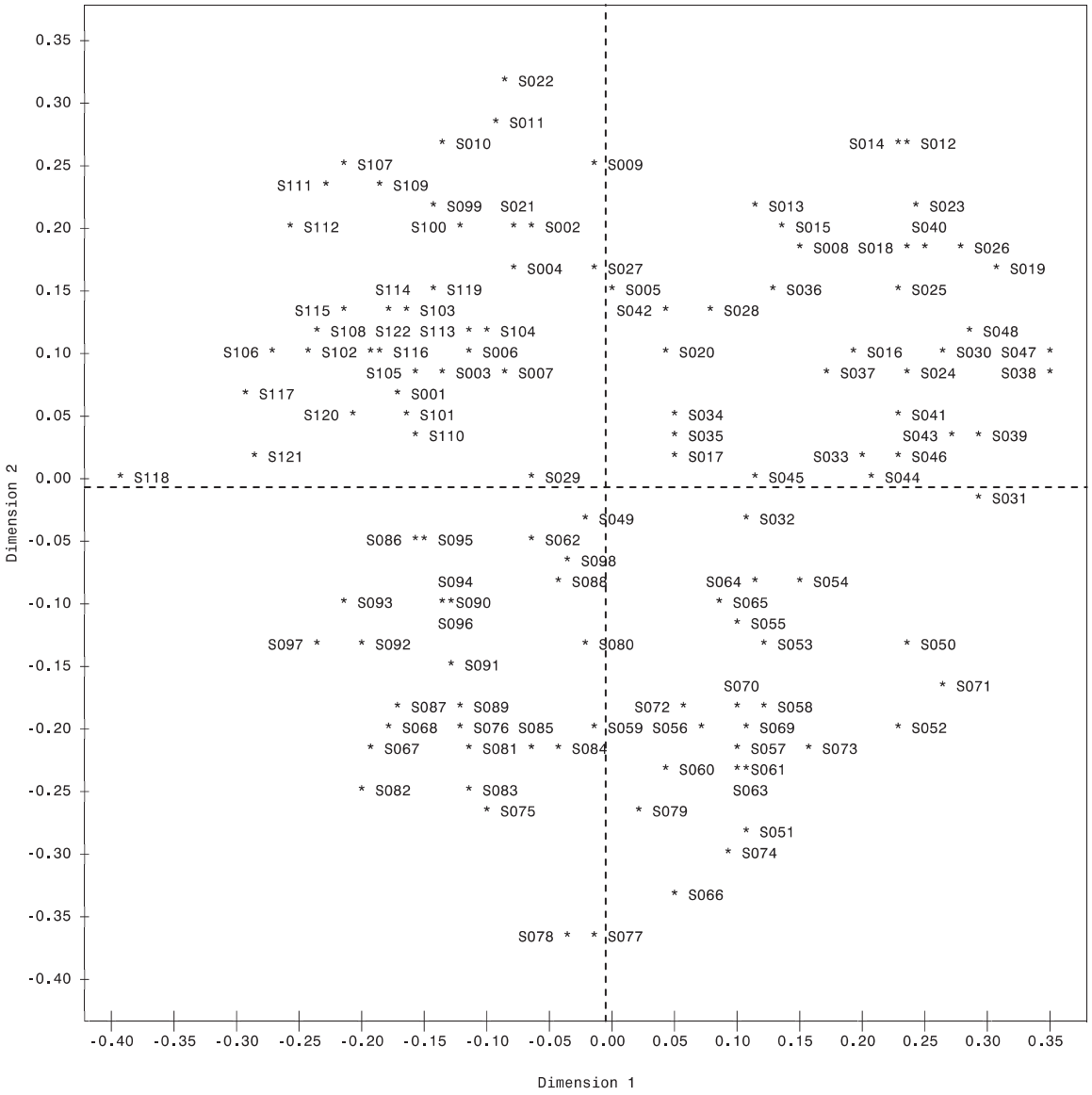


Fig. 2. Two-dimension multidimensional scaling scatter plot of the 122 lines with SCN resistance. Distance estimates are based on the genetic dissimilarity coefficient of 566 SSR allelic fragments. Codes for each PI number can be found in Table 1.



were significantly different from 0. This is another indication that all 7 clusters were distinct from each other. Cluster I had the largest mean value of F_{st} with other clusters, suggesting it was the most distinct cluster.

Comparisons of percentage of accessions resistant to various SCN races among clusters

Cluster I had fewer than 35% accessions resistant to any of the 5 tested races (Races 1, 2, 3, 5, and 14) (Table 5). In cluster II, fewer than 50% of accessions were resistant to any of the tested races with the exception of race 3; more than 70% of accessions in cluster III were resistant to races 1, 3, and 5 and over 50% of the accessions were resistant to race 14. Sixty-five percent of the accessions in cluster IV were resistant to race 3, but fewer than 35% of accessions were resistant to any of the other tested races. Cluster V had a very high percentage of accessions resistant to races 3, 5, and 14 (87.3%, 87.5%, and 81.5%, respectively) and a relatively high percentage (62.5%) of accessions resistant to race 2. In cluster VI, 66.7% and 55.6% of accessions were resistant to races 3 and 5, respectively, but fewer than 40% of accessions were resistant to any other races. In cluster VII, 71.4% of accessions were resistant to race 14, but fewer than 40% of accessions were resistant to any other races (Table 5).

Discussion

The 85 SSR markers used in this study were evenly spread over the 20 linkage groups on the soybean consensus map. Therefore, these markers better represented the whole soybean genome than the RAPD markers used by Chen and Nelson (2004) and the RFLP markers used by Zhang et al. (1999). The 85 SSR loci had an average of 6.7 alleles among 122 genotypes tested (Table 2), which is greater than the 3.1 fragments per marker reported by Chen and Nelson (2004) and the 5.2 alleles per RFLP locus in the study by Zhang et

Table 3. Analysis of molecular variance (AMOVA) for 122 genotypes and 7 clusters assigned by Ward's minimum variance methods based on 566 SSR fragments.

Source of variation	DF	Sum of squares	Variance components	% of variation	*P* value[a]
Among clusters	6	662.1	2.43	8.14	<0.0001
Among individuals within clusters	115	3150.8	0.04	0.13	<0.4946
Among individuals	122	3333.5	27.32	91.73	<0.0001
Total	243	7146.4	29.79	100.00	

[a]Significant test based on 1023 permutations.

Table 4. Pairwise comparisons among clusters (assigned based on Ward's minimum variance) based on F_{st} value for the 122 soybean lines.

	Population pairwise *Fst* value[a]					
Group	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster II	0.09					
Cluster III	0.15	0.06				
Cluster IV	0.13	0.06	0.08			
Cluster V	0.17	0.10	0.09	0.07		
Cluster VI	0.14	0.07	0.10	0.07	0.06	
Cluster VII	0.14	0.08	0.10	0.07	0.10	0.05

[a]All values are significant at the 0.01 probability level.

Table 5. Percentage of accessions resistant to various races among clusters.

		Race 1		Race 2		Race 3		Race 5		Race 14	
Group	No. of lines in cluster	No.	%	No.	%	No.	%	No.	%	No.	%
Cluster I	6	2	33.3	1	16.7	2	33.3	1	16.7	1	16.7
Cluster II	21	5	23.8	2	9.5	12	57.1	9	42.9	9	42.9
Cluster III	21	15	71.4	8	38.1	16	76.2	16	76.2	11	52.4
Cluster IV	26	5	19.2	3	11.5	17	65.4	4	15.4	9	34.6
Cluster V	16	2	12.5	10	62.5	14	87.5	14	87.5	13	81.5
Cluster VI	18	7	38.9	6	33.3	12	66.7	10	55.6	5	27.8
Cluster VII	14	3	21.4	1	7.1	5	35.7	3	21.4	10	71.4
Total	122	39	32.0	31	25.4	78	64.0	57	46.7	58	47.5

al. (1999). The amount of variation (79.5%) explained by the first 2 dimensions in MDS analysis was much higher than that in the study by Zhang et al. (1999), in which only 21% of the total variation was accounted for by the first 2 dimensions in the principal component analysis based on 501 RFLP probe–enzyme combinations. The SSR data are better than the RAPD and the RFLP data for evaluation of the genetic diversity for soybean in terms of efficiency and representation of the entire genome.

AMOVA procedure has been used to analyze genetic diversity in a variety of crops including sweet potato (*Ipomoea batatas* (L.) Lam.; Zhang et al. 2004), sesame (*Sesamum indicum* L.; Ercan et al. 2004), maize (*Zea mays* L.; Reif et al. 2003), and olive (*Olea europaea* L.; Belaj et al. 2002), as well as soybean (*Glycine max* (L.) Merr.; Chen and Nelson 2005). Both AMOVA and clustering analysis were used to quantify the genetic variation among 122 lines in this study; however, they each emphasize a different perspective. AMOVA estimates variance differences among individuals and populations, whereas clustering analysis only estimates the relatedness of genotypes based on genetic distances. In this study, the results from AMOVA demonstrate that variance differences among individuals within clusters is not significant (Table 3), indicating that SCN resistance sources from the same cluster may have major resistance genes in common and should be avoided in a breeding program to increase the genetic diversity of SCN resistance.

Five of the 7 Ward clusters (III, IV, V, VI, and VII) seem to represent 5 distinct groups of resistance sources to different SCN races (Table 5). In cluster III, 13 of the 21 lines are resistant to races 1, 3, and 5. PI 437654 and PI 438489B in this cluster are resistant to races 1, 2, 3, 5, and 14 (Table 1). PI 404166, PI 404198B, and PI 437690 are resistant to races 1, 2, 3, and 5. This cluster seems to represent the sources of resistance to races 1, 3, and 5. In cluster IV, 18 of the 26 lines are resistant to race 3. Almost all lines in this cluster have resistance to a single race except PI 89772 and PI

90763, both of which are resistant to races 1, 2, 3, and 5. On the other hand, most lines in cluster V are resistant or moderately resistant to 2 or more races. In cluster VI, 12 of the 18 lines are resistant to race 3. PI 567336B, PI 567342, PI 494182, and PI 507471 are resistant or moderately resistant to races 1, 2, 3, and 5. These lines are genetically distinct from the other well-known resistant sources such as 'Peking' and PI 88788 and could contain new alleles for SCN resistance. In cluster VII, 10 of the 14 lines are resistant to race 14. In an attempt to improve the association of the clusters with race specificity of SCN resistance, only the markers that are associated with SCN resistance were used in the cluster analysis (17 in total). No improved association could be found (data not shown) using SCN-associated markers only.

Chen and Nelson (2005) have demonstrated that there was a relationship between origin and genetic diversity, especially for accessions from China. The dendrogram generated from this study is useful to examine the relationships between geographical origin and SCN resistance for all PI lines and cultivars used in this research. The clusters, responses to different SCN races, and origins of these lines are summarized in Table 1. Based on this information, cluster I represents a modern cultivar group in which 6 cultivars, 'BSR 101', 'Dunfield', 'Essex', 'Forrest', 'Hartwig', and 'Hill', were included. VARCLUS assigned 5 other cultivars (Hutcheson, Ilsoy, Ina, Kenwood, and Loda) to this group. Based on previous reports, 'Hartwig', 'Hill', 'Hutcheson', and 'Essex' (Anand 1992; Buss et al. 1988; Johnson 1960*a*, 1960*b*; Smith and Camper 1973; Weiss and Stevenson 1955) share some common ancestors. Cluster II was a complex group, but South Korean lines dominated with 7 lines from that country. Cluster III was another complex group where 13 lines originated from China, 2 lines from Japan, 1 line from South Korea, 1 line from Argentina, and 4 lines of unknown origin. Cluster IV represents northern China with 23 lines from China, 1 from Japan, 1 from South Korea, and 1 that is a modern cultivar, 'Pickett 71'. Of the 15 lines from China for which provincial origin is known, 8 are from Shandong. In Cluster V, all of the lines, except 1 unknown, come from China and 12 of the 16 lines from China originated in Jilin. Cluster VI is a complex group, but 8 of the 18 lines originated from Gansu, China. In addition, there were 3 other lines from China, 6 lines from Japan, and 1 line from South Korea. Cluster VII represents the north central region of China with all entries from either Ningxia, Shannxi, Shanxi, or Hebei.

Lines PI 437654 and PI 438489B were the only 2 lines with resistance to most known SCN races among the 122 lines tested in this study. They were introduced from Russia; however, as noted earlier, PI 437654 originally came from China and PI 438489B was purported to have come to Russia from the U.S. The origin of PI 438489B is unknown, but it is very likely also from China. The 2 lines were clustered into 1 group (cluster III; Fig. 1), and were placed in the upper right quadrant of the MDS plot (Fig. 2), indicating that they were related (Fig. 2). However, they were not as closely related as described by Zhang et al. (1999) because the 2 lines were grouped in a different subcluster under cluster III. The relationship of these 2 lines is much closer than reported by Diers et al. (1997), who assigned them to 2 different clusters. These differences are most likely the result of the different types of marker used for estimating genetic distances.

PI 438497 and PI 438496B were introduced from Russia in 1980 with the same name, 'Peking', and were purported to originally be from the US. The American 'Peking' was introduced from Beijing, China, in 1906 and named 4 years later (Bernard et al. 1987). Owing to their similar reactions to SCN races and their sharing the same name, 2 studies were carried out to verify their origins and genetic relationships. Skorupska et al. (1994) and Zhang et al. (1999) used RAPD markers and RFLP markers, respectively, to evaluate these 3 lines and found that PI 438497 was closely related to 'Peking' but that PI 438496B was not. However, all 3 lines were grouped in the same cluster (III) and were placed on the same quadrant (upper right) of the MDS plot in this study (Figs. 1 and 2), indicating that these 3 lines are closely related but not identical.

PI 468916 is a wild soybean and clustered to cluster IV together with 'Pickett 71', a modern cultivar, by the Ward's minimum variance cluster; however, the VARCLUS analysis assigned them to different groups (Table 1). In both cases, the wild soybean accession is grouped with soybean accessions, which is unusual. Other research has shown that with similar clustering procedures RAPD fragment data could consistently separate soybean from wild soybean lines (Chen and Nelson 2004; Li and Nelson 2002). One possible explanation is that 'Pickett 71 inherited considerable marker alleles from its 2 semi-wild ancestors, PI 37335 and 'Peking'. PI 37335 is a very primitive soybean line from China with relatively small seed size (10.8 g/100 seeds) and lower oleic acid concentration (17.1%). 'Peking' has many semi-wild morphological traits such as small seed size, vining growing habit, and low oleic acid concentration (14%).

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